Method Development Studies on the HPLC Analysis of Candesartan Cilexetil using a Core-Shell Column

MaryClare Farrugia [1], Adrian Azzopardi [2] and Claude Farrugia [1]

[1] Department of Chemistry, University of Malta, Msida MSD2080, Malta

[2] Siegfried Malta Ltd., Hal Far Industrial Estate, Hal Far BBG3000, Malta

Introduction

Advances in High Performance Liquid Chromatography (HPLC) have led to increased sample throughput and productivity in pharmaceutical analysis. Recently developed core-shell columns consist of a porous shell and a solid silica core. Thus the mobile phase is only allowed to flow along the porous shell of the particles, decreasing backpressures whilst increasing efficiency.[1] The objective of this study was to use such column technology to minimize the elution time of the drug candesartan cilexetil and its impurities, whilst improving resolution and selectivity.

Methodology

A new HPLC method was developed in order to reduce the initial method run time from 46 minutes to at least 20 minutes, whilst simultaneously improving the resolution. The study was performed on a conventional Waters 2695 Alliance series HPLC apparatus with a 75x4.6 mm Kinetex® 2.6-µm C18 core-shell column. Several methods were developed by an educated trial-and-error approach through variations in mobile phase ratio, gradient methods and mobile phase pH. The method with the best resolution was validated with respect to Impurity C, the most stable of the impurities, to demonstrate suitability for the intended purpose. Statistical analysis was carried out on the results of the tests for accuracy, intermediate precision, stability and robustness.

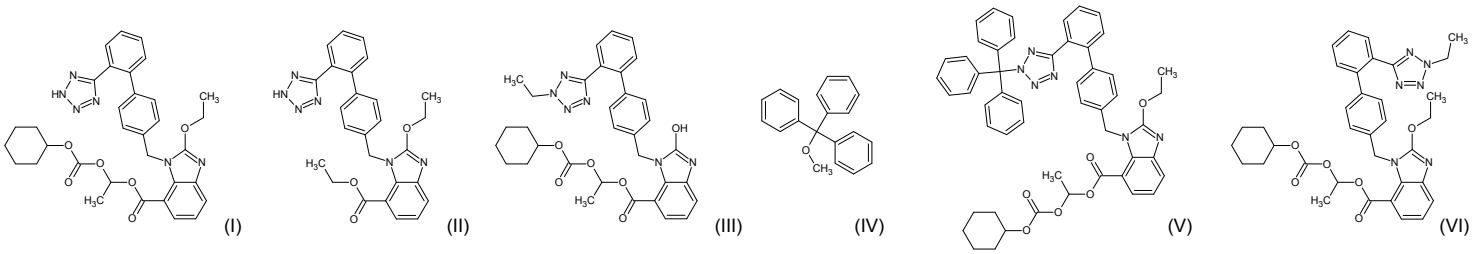

Figure 1 Candesartan cilexetil (I) and its impurities candesartan ethyl ester (Impurity A, II), O-desethyl candesartan cilexetil (Impurity B, III), trityl methyl ether (Impurity C, IV), N2-trityl candesartan cilexetil (Impurity D, V), N2-ethyl candesartan cilexetil (Impurity E, VI)

Results & Data Analysis

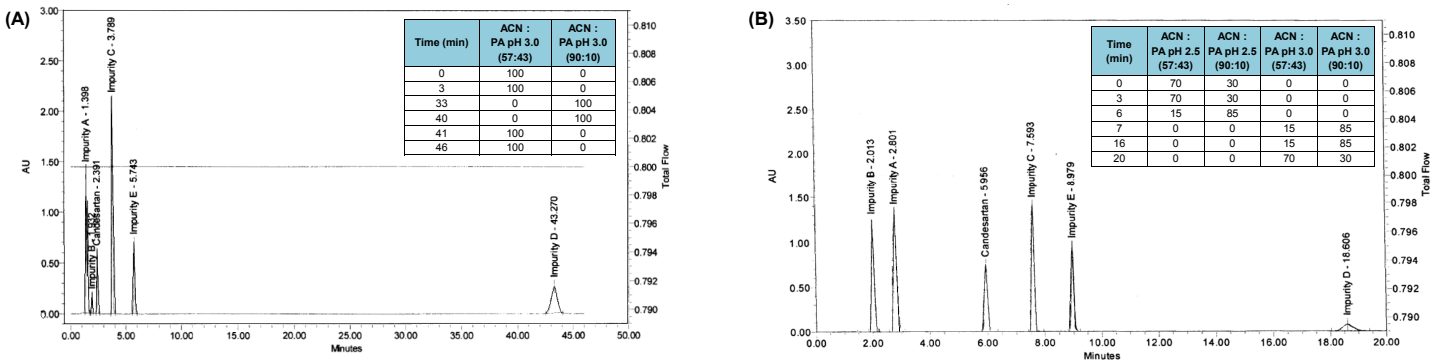

Figure 2 The elution time and resolution of the peaks before (A) and after (B) the method development [ACN: acetonitrile; PA: phosphoric acid]. The new methodology changed from an isocratic to a gradient program method, and involved the use of phosphoric acid at both pH 3.0 and pH 2.5, as against pH 3.0 only prior to method development.

Table 1 The results of the validation tests on the new methodology with respect to Impurity C. All results passed the acceptance criteria.

Validation Test	Test Parameter	Acceptance criteria	Validation test result
Accuracy	Mean % recovery	95 % - 105%	96%
Precision	% RSD	≤ 4.0%	1.68%
Repeatability	% RSD	≤ 4.0%	0.51%
Intermediate Precision	% RSD	≤6.0%	1.98%
Stability	% RSD	Comparison of results	1.30%
Robustness	% RSD	Comparison of results	0.02 < %RSD < 1.06

Table 2 The results of the statistical analysis of the validation tests.

ValidationTest	Statistical Test	Statistical Test Result	Pass or Fail
Accuracy	ANOVA	p-value = 0.039	Fail
Intermediate precision (different days)	Independent sample t-test	t-value = 4.856 p-value = 0.001	Fail
Stability	ANOVA	p-value = 0.039	Fail
Robustness	Three-way ANOVA	various	Pass/Fail

Conclusions

The decrease in retention time of candesartan cilexetil and its impurities was mostly dependent on the ratio of mobile phases in the gradient program, whilst selectivity of the analysis was dependent on the pH of the mobile phase. The peak area of Impurity C, which was used to evaluate the effectiveness of the method, was influenced by the flow rate, pH of the phosphoric acid, and temperature of the column. Although the validation parameters did not pass statistical tests, they were within acceptance criteria and therefore the method was considered suitable for its intended purpose.

References

[1] Koerner, P.; Matthews, T. Increased Efficiency and Resolution with Kinetex® Core-Shell Technology. Phenomenex®, 2009, TN-1058

Acknowledgements

The authors thank Siegfried Generics (Malta) Ltd. for the supply of standard samples and other chemicals, and for the access to scientific equipment in their laboratories.